TEN BROECK — Infantile paralysis

Experiments to Determine if Paralyzed Domestic Animals and Those Associated with Cases of Infantile Paralysis may Transmit this Disease.

By CARL TEN BROECK, M.D.,

With an Introductory Note by Prof. Theobald Smith, M.D.

(from the Department of Comparative Pathology,
Harvard University Medical School,
Boston).

Reprinted from the Forty-fifth Annual Report of the
Massachusetts State Board of Health.

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EXPERIMENTS TO DETERMINE IF PARALYZED DOMESTIC ANIMALS AND THOSE ASSOCIATED WITH CASES OF INFANTILE PARALYSIS MAY TRANSMIT THIS DISEASE.[1]

Introduction.

The sporadic occurrence of poliomyelitis in numerous, apparently unrelated foci, and the tendency of the disease to appear in rural districts which have only slight intercourse with large centers of population, have led to the hypothesis of some animal reservoir for the virus. The incidence of epidemics in summer, when all animal life is most active, favors this hypothesis. On the other hand, poliomyelitis is inoculable only into monkeys and possibly into rabbits. Other species have shown themselves refractory. Even monkeys are not easily infected, and rabbits are as a rule so difficult to infect that their susceptibility is a matter of debate.

There still remains the hypothesis that certain animals may be carriers of the infection without becoming diseased. The infection may vegetate on mucous membranes without invading the central nervous system.

Hypotheses of this sort do not, as a rule, lead anywhere unless as guides to actual experiments which serve to test their validity. The material available for experimentation under the above hypotheses is so abundant that in planning some investigations to trace the virus of poliomyelitis into the lower animals we thought it best to begin with cases of paralysis not explainable as the result of injury, poisons or well-known infectious agents. Before detailing these experiments a brief survey of what is known of animal diseases simulating infantile paralysis will be in order.

There are several spontaneous diseases of animals associated with paralysis that have strong resemblances to infantile paralysis. P. H. Römer [2] discovered a disease among guinea-pigs which causes paralysis and death. It is due to a filterable agent and may be transmitted from guinea-pig to guinea-pig by the intracerebral method of inoculation. It is a meningo-myelo-encephalitis with lymphocytic infiltration.

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[1] By Carl Ten Broeck, M.D., with an introductory note by Prof. Theobald Smith, M.D. (from the Department of Comparative Pathology, Harvard University Medical School, Boston). The cost of this investigation was met by a fund generously contributed by the following gentlemen: Messrs. Frederick S. Converse, William H. Hill, Charles C. Jackson, Charles H. W. Foster, Moses Williame, Moses Williams, Jr., Charles Jackson, Robert Treat Paine, 2d, Frederick P. Royce, Francis R. Bange, and "A Friend." Reprinted from "Infantile Paralysis in Massachusetts, 1907–1912," Massachusetts State Board of Health, 1914; also published in Monthly Bulletin of Massachusetts State Board of Health, July, 1914.

[2] Deutsche Med. Wochenschr., XXXVII., 1911, p. 1209.

M'Gowan and Rettie [1] describe a poliomyelitis in sheep known as "loupin ill," trembling, etc. This disease begins with fever, restlessness, excitability, trembling and muscular twitching, followed by coma and death or by paralysis producing various deformities and often complete loss of the use of the hind quarters.

The lesions of the central nervous system vary according as the animal suffered from the acute or the chronic type of the disease. In the latter, the pia, nerve roots, the gray and white matter and the perivascular sheaths are infiltrated with small round cells. The nerve cells, especially of the anterior horns of the cord, are in various stages of disintegration. Cultures were negative. Intracerebral inoculations of two sheep with brain tissue from acute cases produced no effect.

In the spinal cord and ganglia of a dog affected with paralysis, Flexner and Clark [2] found lesions closely resembling, but not identical with, poliomyelitis lesions as they are found in man and in inoculated monkeys. The lesions, most pronounced in the cervical and lumbar enlargements, consisted of perivascular infiltrations, hæmorrhage, œdema, infiltration and necrosis of the ground substance and necrosis of ganglion cells, which become at times replaced by small round cells.

Inoculation of two dogs and two monkeys (*Macacus rhesus*) led to no positive result. The animals were under observation for several months.

An infectious disease of horses affecting the central nervous system, which may appear both in sporadic and epidemic form and which has been investigated in Saxony, where it has been prevalent for some twenty years, has recently been studied histologically by E. Joest.[3] This author finds much resemblance between the lesions of poliomyelitis and of this horse disease. The disease is at its height in the spring of the year, and its infectiousness is very slight. The affected animal becomes dull and listless, but the paralyses are incomplete.

Joest finds that the pathological changes are chiefly in the brain. There is a marked lymphocytic infiltration of the perivascular lymph spaces, with a tendency to invasion of the nervous tissue. The spinal cord is much less involved.

Although a diplo-streptococcus has been described by several investigators as associated with the lesions, the etiology is not cleared up. Joest describes intranuclear bodies, and is inclined to regard them as belong-

[1] Jour. Pathol. and Bacteriol., XVIII., 1913, p. 47.
[2] Jour. Exper. Med., XVII., 1913, p. 577.
[3] Handbuch d. path. Mikroorganismen, 2d edition, Vol. VI., p. 251.

ing to the chlamydozoa of von Prowazek and to contain the virus of the disease.

Attention has been called to the occurrence of paralysis among domestic animals during epidemics or to individual paralyzed animals that have come in contact with cases in families.

Ed. Müller [1] calls attention to the statements which have appeared in medical writings concerning the possible relation between animals and infantile paralysis (Wickman, Krause, Wilke) and adds one case of his own. A child two years old had carried about and played with a paralyzed fowl. No occurrence pointing to contact either direct or indirect with another case could be discovered.

Bruno [2] gives details of two cases occurring in two children of the same family aged two and three years, respectively. The children had not come in contact with other children, but were restricted to a large garden containing poultry and a few sheep. About six weeks before the disease appeared in the children, the father had purchased a number of ducks from an establishment handling many thousands yearly. Five of the ducks became paralyzed, one died, one was killed and three recovered. Bruno does not hesitate to bring this disease of ducks into etiological relation with the cases of infantile paralysis.

Lust and Rosenberg [3] in studying an epidemic in and around Heidelberg fixed their attention upon paralysis in domestic fowls, which was quite common. They inoculated fowls with a suspension of brain and cord of a paralyzed fowl without success. Four young chickens were placed in a hospital ward with cases of poliomyelitis and fed with nasal secretion of such cases for nine days. This experiment also proved negative. Finally, they injected a strain of poliomyelitis virus into nine chickens, either into the brain or into the peritoneal cavity. This experiment likewise failed to produce the disease.

Neustaedter [4] states that two guinea-pigs contracted poliomyelitis by being kept in proximity to a severe case of inoculation poliomyelitis in a monkey. He also found that in one case swabbing the nasal mucosa with filtered virus from the cord of a monkey caused poliomyelitis in a guinea-pig. No subsequent confirmatory experiments have been reported.

The inoculability of the human virus into the lower animals has received considerable attention. Thus far, the tailed monkeys present the most reliable reaction to this virus after intracerebral inoculation. Rabbits have been tried by various experimenters with uncertain and equivocal

[1] Die spinale Kinderlähmung, Berlin, 1910.
[2] Münch. Med. Wochenschr., 60, 1913, p. 1995.
[3] Münch. Med. Wochenschr., LXI., 1914, p. 120.
[4] Jour. A.M.A., LX., 1913, p. 982.

results. H. K. Marks [1] has more recently gone over this problem again. He found that by using young rabbits, the virus at times may be transmitted through a short series of rabbits and be detected in the last of the series by the use of monkeys. In the experimental work described below, only monkeys were employed, because no other species could be relied upon to yield trustworthy results.

Experimental Part.

Method. — After having been kept under observation for some days the animals were killed, at first, by chloroform, later, as we did not know the effect of chloroform on the virus of poliomyelitis if such should be present on the mucous membranes, by a blow on the head followed by bleeding. A careful autopsy was made and portions of the cord and nasal mucosa removed for inoculation material. In the majority of cases the nasal mucosa was used for inoculation, as it has been shown that in the monkey, at least, the virus persists here long after it can be demonstrated in the cord.[2] We assumed that if the animals were to transmit the disease, they would be more apt to do so through the nose than in any other way that we know of at present. In some cases, however, both nasal mucosa and cord were used for inoculation.

The tissue was ground with sterile sand in a sterile mortar and suspended in salt solution. After standing in the refrigerator over night this suspension was passed through a sterile Berkefeld filter and one or two monkeys inoculated with the filtrate.

Using aseptic precautions and having the monkey under ether anæsthesia, we injected 4 to 6 cubic centimeters into the lateral ventricle. In a few cases we partially sterilized the suspension to be injected, by means of 0.5 per cent. phenol instead of passing it through a filter. The monkeys were kept under observation for at least two months and then, if they remained well, inoculated with tissue from another case, care being taken that it was from a different species than that of the first inoculation, to prevent anaphylactic reaction. In no case was a monkey used more than twice. We lost several monkeys from various causes, as will be seen in the notes, but in all except one case, one of the pair, inoculated from any one animal, lived throughout the incubation period of poliomyelitis. The monkeys used were *Macacus rhesus,* except Nos. 16, 17 and 19, which agreed most nearly with the species description of *Cereocebus galeritus.* The latter were susceptible to poliomyelitis, as we proved by inoculation.

[1] Jour. Exper. Med., XIV., 1911, p. 116.

[2] Flexner and Clark, Jour. Amer. Med. Assoc., 1911, Vol. LVI., p. 585.

In order to show that by our methods we could produce the disease, we made the two following experiments as controls: —

R. L. — Boy, age 4, had a rise in temperature, pulse and respiration, with vomiting. The next day there was a complete paralysis of the left leg, and the knee jerks were absent. On the fifth day he died, showing at this time complete flaccid paralysis of both legs, left arm and partial paralysis of the right arm, marked difficulty in swallowing. Coarse bubbling rales throughout front and back of both lungs. Cord removed and sent to the State Board of Health, July 21, 1911.

The membranes of the cord showed an intense congestion in the lumbar region, and the vessels of the white matter are well marked. Near the cauda equina there is an intense congestion of the anterior horns and of the white matter. Microscopic examination shows the gray matter infiltrated with lymphocytes and red blood corpuscles, the normal tissue being almost entirely destroyed. Clinical and pathological diagnosis: acute poliomyelitis.

Cord suspended in salt solution, filtered and injected into the lateral ventricles of monkeys Nos. 5 and 6, July 22, 1911. Seventeen days later monkey No. 5 became paralyzed in the right leg, and the next day it had a flaccid paralysis of both hind legs. Nine days after monkey No. 6 was inoculated it became very nervous, lost its appetite, and two days later had a paresis of the left arm and both legs. Gradually, it recovered the use of its limbs, and two months after the inoculation was practically normal. Killed at this time, the autopsy showed lumbar cord slightly congested, the superficial vessels slightly prominent and the anterior horns somewhat reddened. No abnormality noted in the remainder of the cord or in the brain. Pleural and peritoneal cavities with contents normal. Microscopical examination shows a marked perivascular infiltration with lymphocytes in the lumbar cord and a degeneration of the anterior horn cells. A piece of muscle removed from the axilla showed marked atrophy.

Monkeys Nos. 18 and 24. — Inoculated Dec. 28, 1912, with 1 cubic centimeter of a 5 per cent. suspension of the cord of a monkey that had died from the effects of an inoculation with a strain of poliomyelitis virus received from the Hygienic Laboratory of the United States Public Health Service at Washington, D. C. Twelve days later these monkeys were found totally paralyzed, and died the next day. Microscopic examination of their central nervous systems showed the typical lesions of poliomyelitis.

Dogs.

Dog No. 57.[1] — Springfield, Aug. 19, 1911. Dog struck by an automobile two weeks ago. Spastic paralysis of hind legs with retention of feces. Autopsy showed a marked lateral curvature of the spine in the upper thoracic

[1] These numbers are running laboratory numbers and have no bearing on the number of cases of paralysis examined.

region with an enlargement of the bodies of the vertebræ. No inoculations made.

Dog No. 58. — From Dr. G., Boston, Sept. 11, 1911. History of paralysis. Dead when received. Slight indication of injections of minute vessels of pia. Post-mortem: putrefaction well under way.

Filtered suspension of cord injected into lateral ventricle of monkey No. 21. Death in a month from a colitis. No evidence of paralysis. Filtered suspension of nasal mucosa injected into lateral ventricle of monkey No. 22. No effects from inoculation after two months.

Dog No. 59. — North Andover, Sept. 22, 1911. Dog had been sick for about a month. Did not use left fore foot. There seemed to be a hyperæsthesia of all limbs. Chloroformed. Autopsy. Walls of stomach and small intestine thickened and congested. Cord apparently normal except in the lower cervical and thoracic regions, where the gray matter appears to be softer than normal. Inoculations not made as there was no apparent connection between this animal and any cases of poliomyelitis.

Dog No. 60. — West Harwich, Feb. 7, 1912. Spastic paralysis of hind legs with retention of urine and feces. First noticed ten days ago. Autopsy. About 10 centimeters above the lumbar enlargement of the cord for a space of about 3 centimeters the cord was found to be slightly larger, firmer and of a more pearly color than the adjacent tissue. On section a grayish white, pearly mass of tissue was seen replacing the gray matter and most of the white matter of the cord, leaving only a ring of the latter around the periphery. Microscopical examination showed that this tumor was probably a fibroma. No inoculations made as the paralysis was most probably due to the tumor.

Cattle.

Cattle No. 237. — Fairhaven, Nov. 8, 1911. Cow, six years old. Raised on the farm and was a great pet of the children. No cases of poliomyelitis for some years in Fairhaven. Two weeks ago it was noticed that there was a slight incoördination in the cow's movements. This increased until there was a complete paralysis of the hind quarters. Killed and autopsied by M. J. Curran, M.D.V., New Bedford, who reports ascites of the abdominal cavity, organs normal, and no signs of tuberculosis. Cord removed and sent to the State Board of Health.

Cord shows slight congestion. Suspended in salt solution, filtered and injected into the lateral ventricles of monkeys Nos. 18 and 22. Monkey No. 22 became blind and was killed a month and a half after the inoculation. Autopsy showed an internal hydrocephalus. Monkey No. 18 remained well for over two months following the inoculation.

Cattle No. 241. — Sharon. Case obtained by kindness of Dr. Mulvehill, March 1, 1912. Heifer, one year old. Complete motor paralysis of the hind quarters beginning four days ago. No known injury and no evidences of external injury. Autopsy. Distal 15 centimeters of lumbar cord bluish, swollen, with an irregular cavity following the lines of the gray matter and

having a hæmorrhagic border. Microscopical examination of the cord shows no evidences of an inflammatory condition, but does show a diffuse hæmorrhage which has probably softened and produced the cavity. No evidence of injury to spine, but Dr. E. E. Southard, who was consulted, regarded the condition as probably due to trauma. No inoculations made.

Cattle No. 253. — Jan. 8, 1913, from Dr. Langdon Frothingham, three pieces of the fore brain weighing about 20 grams, the medulla and the first portion of the cord.

The brain had been sent in by Dr. Playdon of Reading, Mass., who states that before death the cow had a paralysis of its hind legs and that its head was twisted around as in milk fever. Negri bodies were not found by Dr. Frothingham.

The material was received in a putrid condition, so that microscopic examination was not attempted. The portions of brain and cord were ground in a mortar with sterile sand and suspended in 100 cubic centimeters of salt solution. The suspension was shaken for one hour in a machine, frozen and thawed three times, then centrifuged and the supernatant fluid passed through a Berkefeld filter.

Monkey No. 46 received 4 cubic centimeters of the filtrate into its right lateral ventricle and 42 cubic centimeters into its peritoneal cavity.

Monkey No. 58 received 5 cubic centimeters of the filtrate into its right lateral ventricle.

Monkey No. 46 was under observation for six months following the injection and remained perfectly well during this period. One month after monkey No. 58 had been inoculated, it developed a marked diarrhœa. This disappeared for a time, but reappeared about a month later, and three months after the inoculation the monkey died apparently from this chronic diarrhœa. Microscopical examination of the cord showed normal nerve cells and no perivascular infiltration.

Swine.

Swine, No. 101. — Woburn, Aug. 18, 1911. This pig comes from a neighborhood where there is a case of acute poliomyelitis. Along with four others, this pig was taken sick last winter. The nature of the sickness was not determined, but it was stated that during July there was a similar illness among the pigs, from which over a hundred died. Examination shows that the fore legs are used normally while the hind legs are totally paralyzed. Chloroformed and autopsied. Pig about 100 centimeters long and weighing about 80 to 100 pounds. Viscera in general normal, with the exception of the stomach mucosa, of which the fundus is marked by congestion and is pigmented. Slight erosions around margins of cardiac expansion. No parasites. A portion of the ileum near valve congested. Rectum for a distance of 15 centimeters uniformly distended with dry feces into a cylindrical mass about 3 centimeters in diameter. Urinary bladder well distended into a globular mass equal to two fists put together. Spinal cord at level of lumbar enlargement has a distended vein running along its dorsal aspect; several

similar veins on ventral aspect. No other abnormalities noticed. The vertebræ in this region sawn through, but nothing unusual found. Microscopical examination negative.

Injected the filtered suspension of the nasal mucosa into the lateral ventricles of monkeys Nos. 1 and 2. Monkey No. 1 developed an internal hydrocephalus and died in about a month from the time of the inoculation without showing any signs of paralysis. Monkey No. 2 showed no effects from the inoculation after three months.

Swine No. 105. — North Dana, Feb. 20, 1912. One of five pigs that were taken sick last spring, the others dying. Examination shows a paralysis of the extensor muscles of the hind legs. Given morphine and bled to death. Autopsy showed the cord in the lower lumbar region to be possibly a trifle more moist and softer than normal. Microscopical examination negative.

The suspension of the nasal mucosa partially sterilized by means of 0.5 per cent. phenol, injected into the lateral ventricles of monkeys Nos. 32 and 33. No effect from the inoculation during the following two months.

Swine No. 106. — Westwood, Feb. 8, 1912. This pig came originally from a family where there was a case of acute poliomyelitis. Pig taken with an acute illness and died in twenty-four hours. Unable to use legs, which were hyperæsthetic and somewhat œdematous. Autopsy: white female pig weighing about 75 pounds. Post-mortem: decomposition advanced. Bloody around snout. The membranes of the spinal cord were of a purplish red color, but the individual vessels were not very prominent. In the lumbar region the cord was very soft, and the gray matter could not be distinguished from the white. In the dorsal region the cord was soft, yet the markings were distinct. At no place in the cord was there any apparent congestion. The vessels of the membranes covering the brain were markedly enlarged, but the diffuse reddening of the cord was not present. Peritoneal cavity distended with gas; contains considerable blood-stained fluid. Intestine purplish red in color and distended with gas. Not opened. Spleen not enlarged. Right lung crepitant but firm, not collapsed and dark red in color. Section shows a deeply congested tissue with dark clots in the vessels. Left lung less firm than the right, crepitant and on section of a bright red color.

Frozen sections of the cord showed an exudate of fibrin and polymorphonuclear leucocytes in the meninges, but no perivascular infiltration.

We were not successful in getting the suspension of the nasal mucosa sterile by means of 0.5 per cent. phenol, so that no inoculations were made.

Swine No. 107. — This pig was received May 27, 1912, from Westwood. Three months before a paralyzed pig (No. 106) had been received from the same neighborhood. There was a rather close association with a case of poliomyelitis.

On May 19, 1912, this pig became weak in its fore legs and its hind legs were paralyzed. When received at the laboratory, it was found that the hind legs were in a spastic condition and that the animal could move but could not stand on them. The fore legs were weak. The animal had a rectal

temperature of 107.4 degrees. The pig was killed by a blow on the head. The autopsy showed a rather soft, moist cord, the markings of which were indistinct. The brain was apparently normal except for the congestion due to the blow. The liver, spleen, kidneys and adrenal appeared normal on macroscopic examination. The bladder was distended and the rectum was filled with hard feces. The small intestine was normal. The pancreas was firm and on section numerous bands of a firm, yellowish, soap-like substance were visible. The glands in the groin, under the sternum, at the angles of the jaw and in the mesentery were enlarged. Their cortex was hæmorrhagic, and on section they were light gray in color. The heart and lungs were apparently normal.

Microscopic examination of the tissues of this pig showed an early lymph-node tuberculosis, fat necrosis of the pancreas, together with many focal cell accumulations of lymphocytes under the capsule of the adrenal and around the perilobular veins of the liver. There were numerous, relatively large, focal cell collections in the cortex of the kidneys which crowded upon and compressed the tubules. The cells were of the endothelial type. A few mitoses were seen. Necrosis absent. Most remarkable of all, a very evident perivascular lymphocytic infiltration of the vessels of the lumbar cord. There was no destruction of the nerve cells nor accumulation of lymphocytes in the anterior horns of the gray matter.

Portions of the cord of this pig were placed in 0.5 per cent. phenol and kept in the refrigerator until Nov. 8, 1912 (five and one-third months), when they were washed, suspended in salt solution, and the suspension passed through a Berkefeld filter.[1] Five cubic centimeters of this filtrate were injected into the lateral ventricle of the brain of monkey No. 43 and 50 cubic centimeters into the peritoneal cavity of monkey No. 44. Both animals remained perfectly well throughout the following four months.

Swine No. 114. — This pig was received Oct. 25, 1913, from Framingham. White male pig weighing about 50 pounds. Owner had previously killed one paralyzed pig. This one eats normally. Limbs so weak that animal lies down, but is able to walk to reach his food. Reflexes present. Killed after a week's observation, by cutting vessels of neck after stunning with a blow. All the viscera were normal. There were some fresh small hæmorrhages in muscles of back, probably result of death struggle. Some were found in psoas and muscles of diaphragm. Slight hæmorrhagic infiltration of lymph sinus, lymph nodes of neck, mediastinum, aorta and pelvis. Microscopic examination of muscular tissue fixed and sectioned shows hæmorrhages in the septa between bundles of fibers. Red corpuscles well preserved. No cellular infiltration or other signs of inflammation. The cord of this animal was ground up with sand, suspended in five parts of salt solution by weight, shaken and refrigerated for one day; filtered through

[1] Flexner, Clark and Amoss found that the cord of a child which had died of infantile paralysis, kept for fifteen months in 0.5 per cent. phenol, produced typical paralysis when injected into monkeys (Jour. Exper. Med. XIX., 1914, p. 205).

Berkefeld filter. Filtrate in refrigerator for two days, then injected into monkey No. 63, 2.3 cubic centimeters intracerebral, and 20 cubic centimeters into abdominal cavity. Monkey well after four months.

Fowls.

Fowl No. 25. — Lexington, Aug. 10, 1911. This fowl was received from a family where there is a case of acute poliomyelitis. The only sick chicken in the flock. Stands erect with its sternum pushed out and sways from side to side. Falls over when touched, probably from weakness. No paralysis. Feathers around anus soiled. August 12, dead. Autopsy shows brain and cord normal. Anterior nares plugged with a light yellow fibrinous exudate. Eyes moist but without exudate. Nothing abnormal found in the pleural or peritoneal cavities. Diagnosis: avian diphtheria.

Filtered suspension of nasal mucosa injected into the lateral ventricles of monkeys Nos. 9 and 10. No effect from the inoculations in the following two months.

Fowl No. 26. — Woburn, Aug. 25, 1911. This fowl comes from a flock where two or three chickens are said to have died in a mysterious manner, and where the man who cared for them had an attack of acute poliomyelitis that caused his death. Chicken walks with a limp, as though its foot had been injured. No evident paralysis. Killed by a blow on the head. Autopsy showed fatty degeneration of the liver but no other abnormality.

The filtered suspension of the nasal mucosa was injected into the lateral ventricles of monkeys Nos. 19 and 20, and the filtered suspension of the cord into a lateral ventricle of monkey No. 18. All monkeys remained well during the following two months.

Fowls Nos. 27, 28 and 29. — These three fowls were received Sept. 1, 1911, from Boxford. The principal trouble, as far as their walking went, was an apparent loss of equilibrium. When first received, they would stagger when they walked, but later any movement would cause them to fall forward on their heads. After falling they would lie for some time. There was no evident paralysis. For three years chickens on this farm have had some difficulty in walking which appeared when they were about three months old and which progressed until they died. They have evidently had good care and the owner cannot account for their condition.

Fowl No. 27 was chloroformed September 11, when it was nearly dead. Filtered suspension of nasal mucosa was injected into the lateral ventricle of monkey No. 24; filtered suspension of cord injected into the lateral ventricle of monkey No. 23. Both monkeys remained well during the following three months.

Fowls Nos. 28 and 29 were, on Oct. 24, 1911, bled to death, and the nasal mucosæ of both suspended in salt solution, filtered and injected into the lateral ventricle of monkey No. 17. The brains and cervical cords of the two fowls were suspended in salt solution, filtered and injected into the lateral ventricle of monkey No. 16. The monkey showed no effects from the inocu-

lation during the following two months. Immediately after monkey No. 17 had been inoculated, it was totally paralyzed. A little later it had some convulsions, and the next day it had a left-sided hemiplegia. Two days later it died, evidently from some accident during the operation, the nature of which could not be determined at autopsy.

Fowl No. 30. — Leominster, Sept. 23, 1911. This fowl comes from a flock where there have been several cases of paralysis among the chickens, and where the boy who was intimately associated with them has an acute attack of poliomyelitis. The chicken showed a complete paralysis of the legs, which by October 3 had somewhat improved, but which still prevented it from walking. Bled to death and autopsied. Nothing abnormal found.

Filtered suspension of nasal mucosa injected into the lateral ventricle of monkey No. 26. Well for the following two months. Filtered suspension of cord injected into the lateral ventricle of monkey No. 25. Eight days later the monkey succumbed to an attack of colitis and nephritis.

Fowl No. 40. — Ipswich, March 17, 1914. A very fat adult Plymouth Rock hen. The fowl is unable to stand on its feet apparently on account of a paralysis of the muscles of the back. No atrophy of the muscles can be demonstrated. The toes are flaccid, but are moved by the hen. Both legs seem equally involved. The knee jerks are present and equal. The crossed knee jerks are marked. There is no wing drop and the head is held normally. The comb and feathers are in good condition and no ectoparasites can be demonstrated.

This fowl has not been associated with a case of poliomyelitis and there are no other cases of paralysis among the chickens of this flock, though they have occurred in the flock of the breeder from whom this chicken originally came.

On March 21 the fowl was chloroformed and bled to death. There is no atrophy of the muscles of the back or legs and no evidence of an injury to the spine. The abdominal and thoracic viscera and the central nervous system appear to be normal.

Histological examination of the cord at different levels showed a marked perivascular cell infiltration in the gray matter of the lumbar cord in the pia and, to a slight extent, in the white matter. The same condition was found in sections of the dorsal region, but not so pronounced. None was seen in a section of the cervical region and of the midbrain. The infiltrating cells were of lymphoid type. There was no evidence of neuronophagia, but a small number of the nerve cells were swollen, the Nissl bodies absent, and the cytoplasm very attenuated, almost free from stain. Sections from the cords of fowls Nos. 26 and 27, and one other not referred to above, showed no such changes.

The cord, brain and nasal mucosa were ground with sterile sand and suspended in 75 cubic centimeters of salt solution. The suspension was shaken for one hour and placed in the refrigerator for forty-eight hours. It was then centrifugalized and the supernatant fluid passed through a Berkefeld filter.

On March 24 monkeys Nos. 63 and 64 were each given an intracerebral (intraventricular) injection of 5 cubic centimeters of the filtrate and an intra-abdominal injection of 20 cubic centimeters of the same.

Four days after the inoculation both monkeys were very excitable and had some difficulty in climbing. This lasted for a few days and then gradually passed away, leaving the monkeys apparently normal. At no time was there a definite paralysis. Monkey No. 63 is still normal two months after the inoculation, but monkey No. 64 died suddenly six weeks after the inoculation without showing any paralysis or any other symptoms of note. A very careful autopsy failed to reveal any abnormalities, except slightly congested lungs and a very soft, slightly enlarged spleen. From this last organ a bacterium was obtained which has not been classified, but which is not pathogenic for guinea-pigs. Sections of the cord of this monkey show normal nerve cells and no perivascular infiltration.

Horses.

Horse No. 183. — Aug. 4, 1911. Diphtheria-antitoxin horse has had a drooping of the left eyelid and left side of the lip along with a paralysis of the left fore leg for the last month. Chloroformed. Autopsy showed a blood clot in the Sylvian aqueduct ¼ by 1 inch in size.

The filtered suspension of the nasal mucosa was injected into the lateral ventricles of monkeys Nos. 7 and 8. Both monkeys remained well during the following two months.

Horses Nos. 205, 206 and 208. — These three horses in the same stable in Medford were taken sick in March, 1912, at about the same time with fever, difficulty in swallowing, and a paresis of the legs which made it necessary to suspend them in slings. In Wakefield there was a similar disease among the horses belonging to a man who, along with the owner of the Medford horses, had bought some frozen potatoes, which had been fed in both stables. This was the only known connection between the two places and there was no known association with poliomyelitis. Dr. Playdon of Reading reports that some frostbitten potatoes fed to his rabbits caused similar symptoms, with death in four out of the five animals affected. The disease in the horse was also very fatal, as four of the Wakefield and three of the Medford horses died.

Horse No. 205. — This horse died during the night of March 22–23. Autopsy, March 23, 1912, several hours post-mortem. In the mucosa of the small intestine were found a few hæmorrhagic spots 0.5 centimeter in diameter. The lungs showed a marked pneumonic process on the right side. Nothing else of note was found in the peritoneal or pleural cavities. On opening the skull, considerable cloudy yellow fluid escaped. The vessels of the brain were prominent, and in the region of the left Rolandic fissure over an area 5 centimeters in diameter the cortex was of a decided pinkish hue. Similar areas of less extent were present in other places on the surface of the cerebrum. Nothing abnormal was found in the brain on section. The cord in the region of the fourth, fifth and sixth cervical vertebræ was ap-

parently normal. Microscopic examination showed a slight hæmorrhagic exudate in the meninges of the brain and cord.

The nasal mucosa was treated with 0.5 per cent. phenol, suspended in salt solution, and injected into the lateral ventricles of monkeys Nos. 36 and 37. Both monkeys remained well during the following two months.

Horse No. 206. — Autopsy, March 26, 1912, eighteen hours post-mortem. Marked pneumonia of both lungs. Other viscera normal. Considerable blood-stained fluid surrounded the brain and the surface of the latter was congested, but nothing abnormal was found on section. The cord in the region of the fifth cervical vertebra showed nothing more than a congestion of the membranes. Cultures made from the fluid surrounding the brain showed a variety of organisms which were not pathogenic to mice. Microscopic examination of the central nervous system showed a congestion of the vessels of the cerebral cortex but no signs of an inflammatory reaction. No inoculations were made as the disease was regarded as identical with that of horse No. 205.

Horse No. 208. — This horse died April 9; autopsy, April 10, 1912. Cord and brain packed in ice and sent to the laboratory. The brain showed a marked surface congestion, but was otherwise apparently normal. One portion of the cord was apparently normal, while continuous with it was a region where the membranes were markedly congested and the substance of the cord softened. Microscopic examination of the tissues was unsatisfactory on account of the decomposition that had taken place before fixation. There was a marked hæmorrhage into the membranes but no signs of inflammation. In some portions of the cord there was a loss of tissue which involved the gray matter, but this was probably due to the poor fixation.

The cord was treated with 0.5 per cent. phenol, suspended in salt solution, and injected into the lateral ventricles of monkeys Nos. 38 and 39. Both monkeys remained well during the following two months.

Cats.

Cat No. 28. — Boston, May 26, 1911. Cat was picked up on the street. Flaccid paralysis of both hind legs with retention of urine and feces. Chloroformed June 3. Autopsy showed two small hæmorrhagic spots in the dorsal region of the cord. No apparent injury to the spine. Microscopical examination negative.

Filtered suspension of nasal mucosa injected into lateral ventricle of monkey No. 1. Filtered suspension of cord and medulla injected into lateral ventricle of monkey No. 2. Both monkeys appeared perfectly well during the following two and a half months.

Cat No. 29. — Fitchburg. On June 12, 1911, the cat was noticed to favor the left fore leg, and some heat and tenderness were found below the elbow. On June 16 he had a fit and on examination it was found that he could not use either fore leg.

Received at the laboratory Aug. 12, 1911. Examination showed a paralysis of the extensor muscles of both fore legs so that they were sharply flexed

at the elbow. Cat ate and felt well during the time it was under observation. August 23, no change in its condition. Killed by a blow on the head. The autopsy showed gray matter of cord hæmorrhagic in the cervical region. (This may have been due to the blow on the head.) Elbow joints freely movable and apparently normal. No visible atrophy of muscles.

Filtered suspension of nasal mucosa injected into the lateral ventricles of monkeys Nos. 16 and 17. Neither monkey showed any effects from the inoculation during the following two months.

Cat No. 31. — Oct. 16, 1911, from Dr. M. F. Hoar of Fall River. Cat was well but had been associated with a case of acute poliomyelitis in a child. Killed by a blow on the head.

Filtered suspension of the nasal mucosa injected into the lateral ventricles of monkeys Nos. 7 and 8. Monkey No. 8 remained well for over two months following the inoculation. A month after monkey No. 7 was inoculated it became blind, but showed no paralysis. Autopsy showed a marked internal hydrocephalus.

Cats Nos. 32 and 33. — Oct. 16, 1911, from Dr. M. F. Hoar of Fall River. These healthy cats came from a family where there was a case of acute poliomyelitis in a child. Killed by a blow on the head. Filtered suspension of the nasal mucosa injected into the lateral ventricles of monkeys Nos. 9 and 10. Monkey No. 9 remained well for over two months following the inoculation. Nearly a month after monkey No. 10 was inoculated, he stopped eating and in another two weeks was very weak, but showed no signs of any paralysis. Autopsy showed a thickening of the walls of the colon with an enlargement of the mesenteric glands. Central nervous system normal.

Cat No. 34. — Chelmsford, March 28, 1912. Cat has been the pet of a child who is affected with acute poliomyelitis. Shows no signs of disease. Killed by a blow on the head. Suspension of nasal mucosa treated with 0.5 per cent. phenol, injected into the lateral ventricles of monkeys Nos. 34 and 35. Monkey No. 34 was well for over a month and then died from an attack of colitis. Monkey No. 35 was well during the following two months.

Cat No. 35. — Amherst, Oct. 5, 1912. This animal was sent to the laboratory with the history that it had had a bad cough and that it had been intimately associated with two children who were suffering from acute poliomyelitis. As the cat had been introduced into the family about three weeks before the onset of the disease in the children there seemed to be a possibility that it might have brought the infection to them.

The cat was under observation in this laboratory for a month and showed no symptoms of paralysis, cough or loss of appetite. It was killed by a blow on the head and the organs appeared normal on gross and microscopical examination.

The nasal mucosa was placed in 0.5 per cent. phenol for twenty-four hours, washed, ground with sand, and suspended in sterile salt solution. After standing over night in the refrigerator, the suspension was passed through a sterile Berkefeld filter.

Five cubic centimeters of this filtrate were injected into the lateral ventricle

of monkey No. 47. The monkey was under observation for ten months and remained perfectly well throughout this period.

Forty-five cubic centimeters of the filtrate were injected into the peritoneal cavity of monkey No. 48. This animal remained well for two months and then developed a diarrhœa and died seventy days after the inoculation from an acute colitis.

Cat No. 36. — Amherst, Nov. 6, 1912. This animal comes from a house about one-quarter mile away from the one where cat No. 35 lived and where there were two cases of poliomyelitis.

This cat was not a pet and spent most of its time around the barn. For the past few months the animal has appeared ill, but has been around the place as usual. One week before it was shipped to the laboratory, a paralysis of the hind legs was noticed.

The animal was received late in the evening and the next morning was found dead, so that no clinical observations were made on it in this laboratory.

Autopsy shows an extensive pneumonia accompanied by a purulent exudate from the nose. The abdominal viscera are apparently normal. The brain and cord on gross examination are normal except in the thoracic region, where the cord appears to be softer and more moist than normal.

Microscopical examination shows an acute bronchopneumonia and an infiltration of lymphocytes in the meninges surrounding the cord.[1]

Portions of the brain and cord of this cat were placed in 50 per cent. glycerine over night, washed, ground up with sand, and suspended in salt solution. This suspension was kept in the refrigerator for three days and then passed through a sterile Berkefeld filter.

Five cubic centimeters of this filtrate were injected into the lateral ventricle of monkey No. 45 and 50 cubic centimeters into the peritoneal cavity of monkey No. 46.

Eight days after the inoculation monkey No. 45 died from an acute colitis, but monkey No. 46 was under observation for a year and failed to show any signs of paralysis or other disturbances.

Cat No. 37. — This cat was brought to the laboratory Dec. 9, 1912, by Dr. S. of Cambridge, who stated that about three weeks ago one of three cats belonging to his family became listless and refused to eat. A few days later it lost the use of its hind legs, dragging them behind it as it walked. This lasted for about three days, after which time it gradually recovered the use of its legs and is now apparently well. The fore legs were not affected and there were no gastro-intestinal disturbances. About eight days from the onset of the disturbance in the first cat, the second cat began to be listless and showed the same symptoms of paralysis as the first one. The cat brought to the laboratory is the third one belonging to the family, and it is supposed to be in the first stages of the disease.

Examination shows an adult yellow and white castrated cat in good con-

[1] From later studies made on cats it seems probable that this animal was suffering from an infection with bacillus bronchisepticus. In dogs an infection with this organism may be followed by a paralysis, but we have not observed this in cats.

dition. The animal eats very little and remains quiet in its cage. When placed on the floor, it runs and leaps in an apparently normal manner.

On December 12 the cat appears to be stupefied and refuses to eat. When placed on the floor, it moves in a normal manner. Jan. 4, 1913, the cat is perfectly well again. On this date it was killed by a blow and autopsied. Nothing abnormal found beyond a moderate impaction of the rectum with dry feces and a slight distension of the urinary bladder.

The nasal mucosa of cat No. 37 was removed, ground with sterile sand and suspended in sterile salt solution. After standing in the refrigerator for three days, this suspension was filtered and 5 cubic centimeters of the filtrate injected into the lateral ventricle of monkeys Nos. 55 and 56. Monkey No. 55 received in addition 28 cubic centimeters of the filtrate into its peritoneal cavity. Both monkeys made a good recovery from the ether, but monkey No. 55 died twenty-three days and monkey No. 56 sixteen days after the inoculation. Both showed a generalized tuberculosis and neither showed signs of a paralysis between the time of inoculation and the time of death.

As the symptoms shown by cat No. 37 seemed to be of some significance, another cat was placed with it in the same cage. This second animal was under observation forty days and remained well during this period.

Rats.

Two rats were trapped in a house in Waltham in which a case of poliomyelitis had occurred. One of them died and was in a state of decomposition when received at the laboratory. The other was well.

The nasal mucosa, tongue, brain and cord, heart, spleen, kidney, bladder and portions of the liver, lung and rectum with contents from both rats were ground with sand, suspended in salt solution, shaken for two hours, and placed in the refrigerator. After standing for seven days the suspension was filtered through a Berkefeld filter, tested for sterility, and 6 cubic centimeters injected into the lateral ventricles of monkeys Nos. 61 and 62. The latter also received 30 cubic centimeters into the peritoneal cavity. Both monkeys made a good recovery from the ether and beyond a slight attack of diarrhœa remained well during the following five months that they were under observation.

On Nov. 8, 1913, two barn rats were brought to the laboratory with the message that they had been caught in a house in Worcester where there was a case of poliomyelitis. One rat was dead and on autopsy appeared normal.

The second rat sat "hunched" up in the corner of the cage and was evidently very sick. Chloroformed and autopsied. All four feet were very much swollen as a result of œdema. The viscera appeared normal.

Portions of the central nervous system, nasal mucosa, tongue, heart, lungs, spleen, liver, kidney, large intestine with contents and the urinary bladder of both rats were ground with sterile sand, suspended in 100 cubic centimeters of salt solution, shaken for three hours, and allowed to stand in the refrigerator for six days. The suspension was filtered through a Berkefeld filter, tested for sterility, and 2½ cubic centimeters injected into the lateral

ventricles of monkeys Nos. 65 and 66. Monkey No. 66 received in addition 22 cubic centimeters of the filtrate into the peritoneal cavity.

Both monkeys showed a good ether recovery, but three hours after the inoculation were very sick, lying on the floor of their cage completely prostrated. For the next three days the monkeys refused to eat and were very sick, but after this time, they gradually improved. During the following three and a half months that they were under observation, they appeared perfectly well.

Flies.

Nineteen specimens of *Stomoxys calcitrans* were caught Nov. 23, 1912, in the antitoxin horse stables of the State Board of Health at Forest Hills. They were ground up with sterile sand and suspended in 40 cubic centimeters of salt solution, placed in refrigerator for twelve days, filtered through a Berkefeld filter and 4 cubic centimeters injected into one lateral ventricle of each of two monkeys (Nos. 51 and 52). No. 52 received also 33 cubic centimeters into the peritoneal cavity. Both monkeys were under observation for four months and remained well.

On Dec. 7, 1912, this experiment was repeated with twenty-two *Stomoxys* obtained from the same stable. The filtrate was prepared as above, with the exception that the suspension remained in the refrigerator but five days. Monkey No. 53 received 5 cubic centimeters of the filtrate into a lateral ventricle and 14 cubic centimeters into the peritoneal cavity. The monkey died in forty-three days as a result of caseous (tubercular) pneumonia and miliary tuberculosis.

SUMMARY.

Besides the animals noted above we have received and autopsied eight fowls, two cats and two cows. Some of these showed evidence of injury to account for their paralysis, some were in a marked state of decomposition, or else were not paralyzed and had not been associated with cases of poliomyelitis, so that they were not used for inoculation.

We have received in all forty-eight animals, and material from thirty of these has been injected into monkeys. Of these thirty animals there were four rats, seven fowls, nine cats, three horses, four swine, one dog and two cows. Fifteen were paralyzed, four had a questionable paralysis, and eleven were free from paralysis. Thirteen of these animals had been more or less closely associated with human cases of poliomyelitis, and in the other seventeen no such association was known. *In no case did the monkeys inoculated from any of these animals show any signs of a paralysis or symptoms which would indicate that they were infected with poliomyelitis.* In the cords of those that died no perivascular infiltration with lymphocytes was found nor was there a degeneration of the cells of the anterior horns.

A summary of the inoculations made and the source of the material used is given in the following table:—

Animals furnishing Material to be injected.			Material used (Suspensions).	Site of Injection.	Amount injected (Cubic Centimeters).	Monkey injected (Number).	Result.
Species and Number.	Condition.	Exposed to Infantile Paralysis.					
Dog No. 58.	Paralyzed.	Not known.	Filtered cord.	Lateral ventricle.	5	21	Death; acute colitis.
				Lateral ventricle.	5	22	No effect.
Cattle No. 237.	Paralyzed.	No.	Filtered cord.	Lateral ventricle.	4	18	No effect.
				Lateral ventricle.	4.5	22	Killed; internal hydrocephalus.
Cattle No. 253.	Paralyzed.	Not known.	Filtered brain and cord.	Lateral ventricle.	4	46	No effect.
				Peritoneal cavity.	42		
				Lateral ventricle.	5	58	Death in three months; chronic colitis.
Swine No. 101.	Paralyzed.	In neighborhood.	Filtered nasal mucosa.	Lateral ventricle.	4	1	Death; internal hydrocephalus
				Lateral ventricle.	4	2	No effect.
Swine No. 105.	Paralyzed.	Not known.	Nasal mucosa treated with 0.5 per cent. of phenol.	Lateral ventricle.	4	32	No effect.
				Lateral ventricle.	2	33	No effect.
Swine No. 107.	Paralyzed.	Yes.	Cord treated with 0.5 per cent. of phenol for five and one half months, then suspended and filtered.	Lateral ventricle.	5	43	No effect.
				Peritoneal cavity.	50	44	No effect.
Swine No. 114.	Paralyzed(?).	No.	Cord filtered.	Lateral ventricle.	2.3	63	No effect.
				Peritoneal cavity.	20		
Fowl No. 25.	Avian diphtheria.	Yes.	Filtered nasal mucosa.	Lateral ventricle.	1.5	9	No effect.
				Lateral ventricle.	3	10	No effect.
Fowl No. 26.	Limps but is not paralyzed.	Yes.	Filtered nasal mucosa.	Lateral ventricle.	5	19	No effect.
			Filtered nasal mucosa.	Lateral ventricle.	5	20	No effect.
			Filtered cord.	Lateral ventricle.	4	18	No effect.

Animals furnishing Material to be injected.			Materials used (Suspensions).	Site of Injection.	Amount injected (Cubic Centimeters).	Monkey injected (Number).	Result.
Species and Number.	Condition.	Exposed to Infantile Paralysis.					
Fowl No. 27.	No evident paralysis.	Not known.	Filtered nasal mucosa.	Lateral ventricle.	5	24	No effect.
			Filtered cord.	Lateral ventricle.	5	23	No effect.
Fowls Nos. 28 and 29.	No evident paralysis.	Not known.	Filtered nasal mucosa.	Lateral ventricle.	4	17	Death two days after inoculation.
			Filtered brain and cord.	Lateral ventricle.	4	16	No effect.
Fowl No. 30.	Paralyzed.	Yes.	Filtered cord.	Lateral ventricle.	4	25	Death; acute colitis and nephritis.
			Filtered nasal mucosa.	Lateral ventricle.	4	26	No effect.
Fowl No. 40.	Paralyzed.	No.	Filtered suspension of brain cord and nasal mucosa.	Lateral ventricle.	5	63	Excitable four days after inoculation; recovered.
				Peritoneal cavity.	20		
				Lateral ventricle.	5	64	Excitable four days after and death six weeks after inoculation.
				Peritoneal cavity.	20		
Horse No. 183.	Paralyzed.	No.	Filtered nasal mucosa.	Lateral ventricle.	3	7	No effect.
				Lateral ventricle.	4	8	No effect.
Horse No. 205.	Paralyzed.	Not known.	Nasal mucosa treated with 0.5 per cent. of phenol.	Lateral ventricle.	4	36	No effect.
				Lateral ventricle.	4	37	No effect.
Horse No. 208.	Paralyzed.	Not known.	Cord treated with 0.5 per cent. of phenol.	Lateral ventricle.	4	38	No effect.
				Lateral ventricle.	4	39	No effect.
Cat No. 28.	Paralyzed.	Not known.	Filtered nasal mucosa.	Lateral ventricle.	4	1	No effect.
			Filtered cord and medulla.	Lateral ventricle.	4	2	No effect.
Cat No. 29.	Paralyzed.	Not known.	Filtered nasal mucosa.	Lateral ventricle.	4	16	No effect.
				Lateral ventricle.	4	17	No effect.
Cat No. 31.	Healthy.	Yes.	Filtered nasal mucosa.	Lateral ventricle.	4	7	Death; internal hydrocephalus.
				Lateral ventricle.	4	8	No effect.

Animals furnishing Material to be injected.			Material used (Suspensions).	Site of Injection.	Amount injected (Cubic Centimeters).	Monkey injected (Number).	Result.
Speciee and Number.	Condition.	Exposed to Infantile Paralysie.					
Cats Nos. 32 and 33.	Healthy.	Yes.	Filtered naeal mucosa.	Lateral ventricle.	4	9	No effect.
				Lateral ventricle.	4	10	Death; chronic colitis.
Cat No. 34.	Healthy.	Yes.	Nasal mucosa treated with 0.5 per cent. of phenol.	Lateral ventricle.	4	34	Death; acute colitis.
				Lateral ventricle.	4	35	No effect.
Cat No. 35.	Apparently healthy.	Yes.	Nasal mucosa treated with 0.5 per cent. of phenol, then filtered.	Lateral ventricle.	5	47	No effect.
				Peritoneal cavity.	45	48	Death seventy days after inoculation; acute colitis.
Cat No. 36.	Paralyzed.	Indirectly.	Filtered brain and cord.	Lateral ventricle.	5	45	Death; acute colitis.
				Peritoneal cavity.	50	46	No effect.
Cat No. 37.	No paralysis.	No.	Filtered naeal mucosa.	Lateral ventricle.	5	55	Death; tuberculosis.
				Peritoneal cavity.	28		
				Lateral ventricle.	5	56	Death; tuberculosis.
Rats (Waltham).	No paralysis.	Yes.	Portions of various organs suspended in salt solution and filtered.	Lateral ventricle.	6	61	No effect.
				Lateral ventricle.	6	62	No effect.
				Peritoneal cavity.	30		
Rats (Worcester).	No paralysis.	Yes.	Portions of various organs suspended in salt solution and filtered.	Lateral ventricle.	2.5	65	No effect.
				Lateral ventricle.	2.5	66	No effect.
				Peritoneal cavity.	22		
Flies (Stomoxys calcitrans).	Normal.	No.	Bodies suspended and filtered.	Lateral ventricle.	4	51	No effect.
				Lateral ventricle.	4	52	No effect.
				Peritoneal cavity.	33		
Flies (Stomoxys calcitrans).	Normal.	No.	Bodies suspended and filtered.	Lateral ventricle.	5	53	Death in forty-three days; generalized tuberculosis.
				Peritoneal cavity.	14		